

Sequence of combined positive controls

Control sequence for DNA targets:

CTTTGAAGGTAATTTAGATATGGATAATCGACATTTTGACGATTTTTGGCTTGAGTG  
AGAAATTTTATTTCTATGGTTTAGCAGGGGTTGTATTTATTAGATAAAGAACCAGTG  
ACATATCATTCAAGTTTCTGACAGACGGTAGGGTATTGGCCTATTGTCGTGGCATCC  
TAACTCATTCAATTGAATGAATTGGCCATTTTGTTATAATGAGCCGTCCGCATTGTCCT  
CAGGCATTTCACTTAGTGCATACTCATCTTAAGTTGTTTATCAGGGGTGTCGTTCT  
CTTCTTCAACCGTTTCCAGCGCAGAGTCGAAATGGCTTGGAACCTTGCTCTCGCCA  
AACCTGGCGAAAGACTGTATCATATGGTTCTCATCTATTACAGACAGCTCTATCGGC  
TTATGAAGCAAAGACGGCTCAGGACAACGGTTCCTCCGCGGGCGGTCCCTGCTAGCC  
GGACACCGCTGGCAACATTGATCAGGATTTTTCTGGTGATAAA7ACTGGCGAGACTA  
TTTCAATAACGGCTATTTCCGCATGAGGGGCGAATCACAGATTGAATCTTATGTGTCC  
GCCACCATCTGTTGGTACGTTTGGAAACCGCTGGTGCTTGCGCTTGCTCCAGTCTGCGT  
CTGATTCCAACAGAAGTAATGAGCGCAACGGGACTACCAGGGTATCTAATCCTGCC  
GTATTACCGCGGCTGCTTGCTGCCTCCCGTAGGATTCCCACCGGATCACCAAGCTTG  
GAGAGAAGAACCCTTCATCATGCACCACAAGGTTGGTAATAGCAGTCGGCGGTTTCT  
TTTTGGCGGACAATTGCATGCGATGAATAGGTGGCATGTTGGGCGCTAAACCAGTAG  
AGTCTTCAAAATGAATCACTTGACTCTTCAAAATGGTCTGAAAGCATGAATGAACA  
ACACATTTTACTGCTAACTTTGTTGTAATCCTGCCTGTTGTAATCCTGCTTGTACCGG  
GGATAAACTGGACCACGGTGACAGAAGACAACAAAACCCACCGCCCAATAACGAAT  
TGCCCGATTGAAACGACTTGCTCATCAACTTTCGATGGTACGCTACGTGCTTACCAT  
GGGACAACGGTTAACGGAGAATCAGCCTTTTCCGCGTTCCTTGACCGCCTTTCCGAT  
ACCGTCTCTGCAGCAATACCTCCGGATTCCGTTTGTGCCATCATTTTCTAAGCACCTG  
GTGTCCATCCTGTTTCTATTTGGAGCATTAGGTAATACCACTTCTCGACTGCAAAGA  
CGTATGGCTCTGCAATAGGTAAGTCCAGATAGTGGCACAGGGGATAATTTGTGCCACG  
GTGGGGTTTCTGAACTTCTGCACCAGGCCCGGGCTCAGGATGTGCATGTAAGACCAT  
TGCGGCCACATCGGTGTCTGTTATTAACCTTTGCTGTGGATATACGAGGCTATCAG  
GCGCGTTTTGACCTTCGCTACCAGTGGGACAAGAAAGTGGTGGGACTGGAAGTAC  
GAGAGCCAGTTTGCCGG

Control sequence for RNA targets:

TAATACGACTCACTATAGGGCGCTGGATGCGCTTCCATGAGTGGACAGGAGATCGCG  
TAAATGATGATGGCGTCTAAGCAGTTGCTTGCTGCGTTCATCACAGAAGAGCAACTC  
CATCGCAGAAGTGTGATGGCTAGCAAGCAAGAGCCAATGTTTACAGATGGATGAGGTG  
GGAGGGCGATCGCAATCTTGTGAATGAAGATGGCGTCGAACCATCTACACATGACC  
CTCTATGAGTAGTTAAAAGCTAACACTGTCAAAGCTATAGGGGCGTTATGTGACCGA  
TCAGGCTCTCGCCACCTACTTTGAACAAGCTGTGGCATGCTACCCTGGTTCATAGGT  
GGTACAGCAGCTGGTACATTGGTGGCACTAGTGTGAGATGGAGGGTGGCACTAC  
CCCTCTCCGTATCACTCACATCGATAGATCAAGGTGCCCTACAAGCGTCATCGTGGG  
GTCCGCCGTAC

Two assays were designed to specifically interrogate positive controls for DNA and RNA, respectively, in case that lab contamination of positive controls was suspected. The primer and probe sequences were 5'-CATGAGTGGACAGGAGATCG-3', 5'-TGAACATTGGCTCTTGCTTG-3', FAM-CAACTGCTTAGACGCCAT-MGB for RNA control and 5'-TAAAACGACGGCCAGTGAAT-3', 5'-TCAAGCCAAAATCGTCAAA-3', FAM-CCTCGCGAATGCATCTA-MGB for DNA control.

Supplemental Figure S1 Matrix inhibition test. Three lots of healthy donor stool were spiked with a combined positive control during extraction, then extracted DNA and RNA were analyzed with TAC. For each lot, average Ct value and coefficient variance (CV) were calculated for each target (n=4).

